Chemical Physics Letters 603 (2014) 13–17

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

A study of the hydration of ribonuclease A using densitometry: Effect of the protein hydrophobicity and polarity

Vladimir A. Sirotkin *, Aigul V. Khadiullina

Kazan Federal University, A.M. Butlerov Institute of Chemistry, Kremlevskaya Str. 18, 420008 Kazan, Russia

ARTICLE INFO

Article history:
Received 30 January 2014
In final form 16 April 2014
Available online 23 April 2014

ABSTRACT

The excess volumes of the binary system of ribonuclease A (RNase A) with water were obtained as a function of composition at 25 °C. The excess quantities for RNase A were compared with the published data for several unrelated proteins (lysozyme, serum albumin, lactoglobulin, and chymotrypsinogen A). The hydrophobicity of these proteins is gradually changed over a wide range. It was found that the more hydrophilic a protein is, the more significant the hydrophilic hydration contribution is. RNase A is the most hydrophilic protein in the present study, and it has the most significant hydrophilic hydration contribution.

1. Introduction

Water binding (hydration or biological water) plays a key role in determining the structure, stability, dynamics, and functions of proteins [1–7]. On the other hand, there are essential differences between hydration and the bulk water surrounding a protein [1–9]. Thus, a characterisation of the hydration of proteins requires elucidating the effects of the protein on water and *vice versa*. Therefore, a quantitative estimation of the protein and water contributions to the thermodynamic functions of binary protein–water systems is of considerable importance and practical interest.

Thermodynamic studies have traditionally been very important in ascertaining a better understanding of protein–water interactions. Volume is an important thermodynamic quantity directly associated to the compactness or globularity of a protein, and is generally dependent on a combination of factors [6,9–13]. The hydration of charged and polar groups reduces volume. However, the volume changes associated with the exposure of hydrophobic groups depend on the model compounds selected, ranging from small negative to positive values. Moreover, it is not clear whether the volume change associated with hydrophobic hydration plays an important role in the total volume change.

Several studies have investigated the water density (volume) in the hydration shell of the proteins. A combined neutron and X-ray scattering study [14] showed that the average density of the first hydration shell of proteins is significantly higher than that of bulk water. This finding is consistent with the results obtained from the molecular simulation, crystallographic, and solution studies [10,15–17]. The higher density of preferentially bound water and a comparison of hydration values in solution are also given in Ref. [6].

The partial specific volumes for the majority of globular proteins in aqueous solutions fall within a narrow range between 0.70 and 0.75 $cm^3\ g^{-1}$ [3,6,10–13,18,19]. The concentration dependences of the apparent volumes of serum albumin, ovalbumin, and oxyhemoglobin were measured at high protein concentrations (protein mass fraction, w_2, ~0.3–0.4) [18]. No effect of protein on the solvent was observed at these concentrations, suggesting a constant partial specific volume of the solvent in the studied concentration range. The dilatometric measurements of serum albumin and oxyhemoglobin are consistent with this conclusion [19].

Direct volumetric studies of binary water–protein systems at low hydration levels are relatively rare. Volumes of protein–water systems with a w_1 (water mass fraction) between 0 and 0.56 were measured in Refs. [20–23]. It was shown that at a $w_1 > 0.2–0.3$, the volumes of the binary protein–water systems depend linearly on the water content. However, at the lowest w_1 values, there are deviations from this linearity. Thus, these studies [20–23] suggest that the partial specific volumes of proteins are lower at high hydration levels than in the dried state. Bull and Breese [20] estimated that at a $w_1 < 0.2$, the partial specific volume of water is lower than that of bulk water.

The measured volumes [10–13,18–23] contain total information on the binary water–protein systems, including the corresponding conformational changes in the protein structure and the glass transition. However, the simultaneous estimation of the protein and water contributions to the volume of binary protein–water systems in the entire range of water contents has not been attempted.

* Corresponding author.
E-mail address: vsir@mail.ru (V.A. Sirotkin).

http://dx.doi.org/10.1016/j.cplett.2014.04.023
0009-2614/